

# Introduction to Pathway Analysis and Visualization

## Exercise 1 instructions

The example data files represent lists of differentially expressed genes for ORA and GSEA analysis:

### ORA data files:

**lung.expr.up.txt:**  $\log_2FC \Rightarrow 2$  AND  $\text{adj.P.Value} \leq 0.05$

**lung.expr.dn.txt:**  $\log_2FC \leq -2$  AND  $\text{adj.P.Value} \leq 0.05$

### GSEA data file:

**lung.expr.entrez.gsea.txt:** All genes and associated rank

## Functional Enrichment Analysis

### EnrichR

#### Introduction

1. Go to the **EnrichR** website: <https://amp.pharm.mssm.edu/Enrichr/>
2. To explore available libraries, go to the **Libraries** tab to browse.
3. You can also browse libraries by term, for example “cancer”. From the results, you can download individual gene sets in gmt format.

#### Gene Ontology

1. Open the file with upregulated genes, **lung.expr.up.txt**, and copy the full list of symbols.
2. On the **Analyze** tab, paste the list of gene symbols in the input box.
3. *Note:* There is an option to contribute your gene list, which makes it available to others. If you select this option, you will be prompted to assign a name and provide a brief description of the list.
4. Click **Submit**.
5. When the results load, go to **Ontologies -> GO Biological Process**.
6. By default, the terms are sorted by p-value ranking. Clicking on any of the bars representing terms re-sorts the bar graph by the different score.
7. To save the bar chart, click either of the **SVG / PNG / JPG** buttons just above the bar graph.
8. It is also possible to change the color scheme by clicking the **Settings** icon at the top right.

9. Switch to the **Table** view at the top. To export the table, click the **Export entries to table** link at the bottom.
10. Open the downloaded table. Each row contains statistics for one GO term, and a list of changed genes for that term.

## WikiPathways

1. At the top of the page at EnrichR, click **Pathways** to see results for pathway sets.
2. Click the heading for **WikiPathway 2021 Human**.
3. Again, we can export the bar graph and table.
4. To share or archive the results, a permanent link is available by clicking the link icon at the top of the page.
5. *Optional*: Repeat for the down-regulated list, **lung.expr.dn.txt**.

## WebGestalt

### GSEA Pathways

1. Go to the **WebGestalt** website: <http://www.webgestalt.org/>
2. Select **Homo sapiens** as species, **GSEA** and for Functional Database select **pathway** and then **WikiPathways**.
3. Under Gene List, select **Gene symbol**.
4. Open the **lung.expr.entrez.gsea.txt** file and copy the contents.
5. In the WebGestalt **Gene List** box, paste the GSEA data.
6. Skip the **Advanced Parameters** for now and click **Submit**. The results may take several minutes to load.
7. The top of the results page includes a job summary and a link to download the results (top right).
8. Results are displayed as a bar chart by default. Right-clicking on the bar chart lets you download in either PNG or SVG format. Clicking on the bars updates the pathway-specific display at the bottom of the page. The **Table** and **Volcano plot** views have similar interactivity.